

Claims

1. A method for detecting the presence of a IgG4 polypeptide having a selected disulfide linkage pattern in a sample comprising,
5 loading a sample containing a polypeptide having a selected disulfide linkage pattern, wherein the sample comprises an inhibitor of disulfide bond rearrangement, onto a chip comprising a channel having a separation medium effective to act as an obstacle to the migration of the polypeptide having a selected disulfide linkage pattern, and at least two electrodes disposed within the channel to induce an electric field,
10 applying an electric field across the separation medium of the chip whereby a separation of the IgG4 polypeptide having a selected disulfide linkage pattern as compared to a IgG4 polypeptide not having the selected disulfide linkage pattern is achieved, and
determining the presence of the IgG4 polypeptide having a selected disulfide
15 linkage pattern.
2. A method for detecting the presence of a polypeptide having a selected disulfide linkage pattern in a sample consisting of a mixture of polypeptide multimers having two or more polypeptide chains and comprising at least one disulfide linkage between the
20 polypeptide chains comprising,
loading a sample containing the mixture of polypeptide multimers, wherein the sample comprises an inhibitor of disulfide bond rearrangement, onto a chip comprising a channel having a separation medium effective to act as an obstacle to the migration of the polypeptide having a selected disulfide linkage pattern, and at least two electrodes
25 disposed within the channel to induce an electric field,
applying an electric field across the separation medium of the chip whereby a separation of the polypeptide having a selected disulfide linkage pattern as compared to a polypeptide not having the selected disulfide linkage pattern is achieved, and
determining the presence of the polypeptide having a selected disulfide linkage pattern.
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3. The method of claim 1 or 2, wherein the inhibitor is a sulfhydryl alkylating reagent.
4. The method of claim 3, wherein the sulfhydryl alkylating reagent is selected
35 from the group consisting of iodoacetamide and N-ethylmaleimide (NEM).
5. The method of claim 4, wherein the sulfhydryl alkylating reagent is N-ethylmaleimide (NEM).

6. The method of claim 5, wherein the amount of N-ethylmaleimide (NEM) is between about 1mM to about 10 mM.
- 5 7. The method of claim 1 or 2, wherein the method further comprises determining the presence of a polypeptide impurity.
8. The method of claim 1, wherein the IgG4 polypeptide having a selected disulfide linkage pattern is a half-antibody.
- 10 9. The method of claim 2, wherein the polypeptide having a selected disulfide linkage is a half-antibody.
10. The method of claim 9, wherein the half-antibody is of the IgG4 class.
- 15 11. The method of claim 1, wherein the IgG4 polypeptide having a selected disulfide linkage pattern is recombinantly produced.
12. The method of claim 1 or 2, wherein the polypeptide is recombinantly produced.
- 20 13. The method of claim 1 or 2, wherein the polypeptide having a selected disulfide linkage pattern is recombinantly produced.
14. The method of claim 1, wherein, the IgG4 polypeptide not having the selected disulfide linkage pattern is an anti-integrin antibody.
- 25 15. The method of claim 2, wherein the mixture comprises an anti-integrin antibody.
16. The method of claim 14 or 15, wherein the anti-integrin antibody is
- 30 recombinantly produced.
17. The method of claim 1 or 2, wherein the sample is obtained from the growth medium of a cell culture.
- 35 18. The method of claim 1 or 2, wherein the sample comprises about 1 to about 5000 ug/ml of a polypeptide having a selected disulfide linkage pattern.
19. The method of claim 1 or 2, wherein the separation medium is a gel polymer.

20. The method of claim 1 or 2, wherein the separation medium is non-reducing.
21. The method of claim 1 or 2, wherein the migration of the polypeptide is detected
5 using a fluorescence detector.
22. The method of claim 1 or 2, wherein the electric field is non-alternating.
23. The method of claim 1 or 2, wherein the separation further comprises isoelectric
10 focusing.
24. The method of claim 1 or 2, wherein the separation is according to the
molecular weight of the polypeptide.
- 15 25. The method of claim 1 or 2, wherein the chip comprises a precast gel polymer.
26. A kit for detecting the presence of a polypeptide having a selected disulfide
linkage pattern comprising, a chip and instructions for carrying out the method of claim
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27. A kit for determining the purity of a therapeutic polypeptide having a selected
disulfide linkage pattern comprising, a chip and instructions for carrying out the method
of claim 1.
- 25 28. The kit of claim 26 or 27, wherein the kit further comprises a component selected
from the group consisting of, separation medium, non-reducing buffer, protein dye,
formulation buffer, and means for inducing an electric field through a separation
medium.
- 30 29. The kit of claim 26 or 27, wherein the kit further comprises instructions for
determining the presence of a polypeptide impurity.
30. The kit of claim 26 or 27, wherein the kit further comprises one or more
polypeptide standards.
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31. A method of inhibiting disulfide bond rearrangement, wherein the polypeptide is
incubated with a sulfhydryl alkylating agent selected from the group consisting of
iodoacetamide and N-ethylmaleimide (NEM).

32. The method of claim 31, wherein the sulfhydryl alkylating reagent is N-ethylmaleimide (NEM).
- 5 33. The method of claim 32, wherein the concentration of N-ethylmaleimide (NEM) is between about 1 mM to about 10 mM.
34. The method of claim 31, wherein the disulfide bond rearrangement occurs upon exposure to heat.
- 10 35. A composition comprising a polypeptide and inhibitor of disulfide bond rearrangement, wherein the inhibitor is a sulfhydryl alkylating agent.
36. The composition of claim 35, wherein the sulfhydryl alkylating agent is selected from the group consisting of iodoacetamide and N-ethylmaleimide (NEM).
- 15 37. The composition of claim 36, wherein the sulfhydryl alkylating reagent is N-ethylmaleimide (NEM).
- 20 38. The composition of claim 37, wherein the concentration of N-ethylmaleimide (NEM) is between about 1 to about 10 mM.
39. The composition of claim 35, wherein the polypeptide is a multimeric polypeptide.
- 25 40. The composition of claim 39, wherein multimeric polypeptide is an antibody or half-antibody.
41. The composition of claim 40, wherein the antibody is an IgG4 antibody.
- 30 42. The composition of claim 41, wherein the antibody is an anti-integrin antibody.
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